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Chromosome Numbers of Seven Species of *Sedum* and *Sinocrassula indica* (Crassulaceae) in East Himalaya

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Chromosome numbers of the seven species of *Sedum* and *Sinocrassula indica* collected in East Himalaya were investigated for the first time. The chromosome numbers 2n = 22, 24, 28, 34, 48, 70, and 84 were found in *Sedum* and 2n = 22 in *Sinocrassula indica*. In *Sedum* the basic number is different among the species except for x = 12 shared by *S. triactina* and *S. henrici-roberti*, and x = 14 by *S. multicaule* and *S. magae*. Morphologically the species with common basic number differ greatly and belong to different sections. An intraspecific polyploid series was found in *S. multicaule* in which the plant with 2n = 28 was estimated as diploid and differed from that with 2n = 84 (hexaploid) in having slender forms. The chromosome number 2n = 70 in *S. trullipetalum* is considered to show odd polyploidy, judging from extremely low pollen fertility which is also found in all the herbarium specimens examined. This suggests that the plants of Himalayan *S. trullipetalum* occurring in Nepal and Bhutan are reproduced by apomixis.

Key words: chromosome numbers, Crassulaceae, Himalaya, *Sedum, Sinocrassula*

In Nepal and Bhutan, species of *Sedum* cover a wide range of altitude from the upper evergreen broad-leaved forest zone to the subnival zone, approximate elevation between 2600 m and 5200 m above the sea level, and are major elements of vegetations of outcrops, stony or gravelly slopes. *Sinocrassula indica* occurs in stony places of localized treeless arid areas in central and western Nepal, elevations between 2400 m and 2800 m. In this paper we classified the Himalayan species of subgenus Sedum into four sections, Oreades, Japonica, Bracteata, and Filipes, provisionally. Though the taxonomy of the Himalayan species of *Sedum* is still insufficient, 16 species have been known from Nepal (Hara and Williams 1979). Among them 11 species belong to section Oreades which concentrates in the region from eastern Himalaya to southwestern China.

Morphological variability in *Sedum* is reported to be partly derived from polyploidy and other cytological behaviours (Uhl 1961, Amano 1990), but Himalayan *Sedum* and *Sinocrassula* have not yet been studied cytologically. In the present study we have investigated four species in sect. Oreades, and each one in other three sections of *Sedum* and one species of *Sinocrassula*. Species of sect. Oreades in *Sedum* occur in the alpine and subnival zones above (3650–) 4000 m, while the species outside of sect. Oreades, *S. multicaule* (sect. Japonica), *S. triactina* (sect.

Bracteata), and *S. filipes* (sect. Filipes) occur in the forest zones in elevations less than 3400 m. The present paper aims to publish the chromosome numbers and the chromosome features and discuss chromosomal as well as morphological variations and the correlation between both variations.

Materials and Methods

Materials for the present cytological study were sampled from 27 populations in Nepal Himalaya and one population in Bhutan by six botanical expeditions carried out during 1983 to 1997. Route maps of their expeditions in Nepal are shown in Ohba and Malla (1988), Ohba (1992), Miyamoto (1995), and Wakabayashi (1998).

Root tips used for cytological observations were fixed in the field. After pretreatment made by 0.05 % cochicine solution for about 3 hr, the materials were fixed in Newcomer's fluid. For chromosomal observation, the fixed materials were completely hydrated using an ethyl-alcohol series, and they were soaked in a mixed 1:1 solution of 2 % pectolyase and 4 % cellulase for 1 hr at 37°C in order to macerate cell-wall and to soften the root tips. They were stained with 2 % acetic orcein for about 12 hr and then squashed. The voucher specimens for the present cytological study are kept in the herbaria of the University of Tokyo (TI) and Makino Herbarium, Tokyo Metropolitan University (MAK).

Results

Table 1 shows the chromosome numbers counted from 27 populations of seven species of *Sedum* and a single population of *Sinocrassula indica*, and their localities, elevations, and the voucher specimen number. Figures 1 and 2 show the chromosomes observed in a somatic cell of each species. The description of the cytological features of each species is as follows:

Sedum filipes Hemsl.

2n = 34 (Figs. 1a, a′). All of the three populations examined had the same chromosome number. The chromosomes are very small, 0.6–1.3 μm long at metaphase and gradually increased in size in a complement.

Sedum triactina A.Berger

2n = 24 (Figs. 1b, b′). In all of the four populations examined, the same chromosome number was counted. The chromosomes are 1.0–1.7 μm long at metaphase. One pair of metacentric chromosomes has satellites.

Sedum multicaule Wall. ex Lindl.

2n = 28, 84 (Figs. 1c, c′; d, d′). Of the five populations examined, 2n = 28 has been found in one and 2n = 84 in the other four. In the somatic cells with 2n = 28, only the chromosomes at prometaphase were observed and no clear picture were obtained (Fig. 1c), but one pair of chromosomes seems to have satellites. The chromosomes of 2n = 84 are very small, 0.5–1.3 μm long at metaphase.

Sedum trullipetalum Hook.f. & Thoms.

2n = 70 (Figs. 2d, d′). In all of the six populations examined, the same chromosome number was counted. The chromosomes are small and gradually reduced in size in a complement from 1.5 μm to 0.8 μm long at metaphase.

Sedum oreades (Descne.) R.-Hamet

2n = 22 (Fig. 2c). In all of the five populations examined, the same chromosome number was counted. All the chromosomes in a complement are large as compared with those of the other species, 1.4–2.0 μm long at metaphase.

Sedum magae R.-Hamet

2n = 28 (Figs. 2b, b′). Only one population was examined. The chromosomes are very small, 0.7–1.0 μm long at metaphase.

Sedum henrici-roberti R.-Hamet

2n = 48 (Figs. 2a, a′). In all of the three populations examined, the same chromosome number was counted. The chromosomes are gradually reduced in size in a

Table 1. Chromosome numbers of Himalayan *Sedum* and *Sinocrassula* and the voucher data

Taxa	Chromosome number (2n)	Voucher data: locality, specimen no., altitude in parentheses
Sedum subg. Sedum		
Sect. Filipes		
S. filipes Hemsl.	34	N*: Dhaulagiri zone; Gasa-Lete, 8320258 (2260 m)
	34	N: Gandaki zone; Chame-Dhanagyang, 8320517 (2600 m)
	34	N: Dhaulagiri zone; Tatopani-Ghasa, 8330496 (2000 m)
Sect. Bracteata		
S. triactina A.Berger	24	N: Janakpur zone; Serdingma-Dubikharka, 8520038 (3400 m)
	24	N: Janakpur zone; Neju-Choarma, 8520249 (3200 m)
	24	N: Kosi zone; Gidde-Khokling, 9151055 (3350 m)
	24	B: Wandi Phodrang Dist., Bhja-Maorothang 9361543 (3350 m)
Sect. Japonica		
S. multicaule Wall. ex Lindl.	28	N: Gandaki zone; Chame-Dhanagyang, 8320511 (2650 m)
	84	N: Dhaulagiri zone; Ghasa-Lete, 8320253 (2200 m)
	84	N: Sagarmatha zone; Loding-Pike Bhanjyang, 8520393 (2600 m)
	84	N: Sagarmatha zone; Pike-Pike Khop 9584047 (2530 m)
	84	N: Sagarmatha zone; Taktor-Mopung, 8520280 (2800 m)
Sect. Oreades		
S. trullipetalum Hook.f. & Thoms.	70	N: Janakpur zone; Beding-Na, 8320632 (4050 m)
	70	N: Sagarmatha zone; Thosa Kharka-Beni Kharka, 8520291(4000 m)
	70	N: Sagarmatha zone; Beni Kharka, 8520300 (3950 m)
	70	N: Sagarmatha zone; Hinku Valley, Rangdu Kharka-Horsola Kharka, 9710192 (3820m)
	70	N: Sagarmatha zone; Hinku Valley, Khare-Tangnag, 9710288 (4400 m)
	70	N: Sagarmatha zone; Hinku Valley, Khare-Tangnag, 9710287 (4300 m)
S. oreades (Descne.) R.-Hamet	22	N: Sagarmatha zone; Thosa Kharka-Beni Kharka, 8520295 (4100 m)
	22	N: Sagarmatha zone; Beni Kharka, 8520299 (4000 m)
	22	N: Sagarmatha zone; Beni Kharka-Tschokarma, 8520376 (4600 m)
	22	N: Kosi zone; Goja-Shuwan Kharka, 9151206 (4200 m)
	22	N: Kosi zone; around Topke Gola, 9151231 (3650 m)
S. magae R.-Hamet	28	N: Kosi zone; Topke Gola-Bomrang, 9151241 (3700 m)
S. henrici-roberti R.-Hamet	48	N: Sagarmatha zone; Dudh Kund, 8520331 (4500 m)
	48	N: Sagarmatha zone; around Beni Kharka, 8520307 (4500 m)
	48	N: Janakpur zone; around Thare Og, 8520186 (4500 m)
Sinocrassula indica (Descne.) A.Berger	22	N: Dhaulagiri zone; Kagbeni-Muktinath, 8330343 (2800 m)

*N, Nepal; B, Bhutan.

complement from 2.0 µm to 0.7 µm long at metaphase. One pair of large submetacentric chromosomes has satellites.

Sinocrassula indica (Descne.) A.Berger

2n = 22 (Figs. 2e, e′). Only a single population was examined. The chromosomes are small, 1.0–1.7 µm long at metaphase.

Discussion

The authors have detected the chromosome numbers of seven of the 16 species of *Sedum* known from Nepal and one of

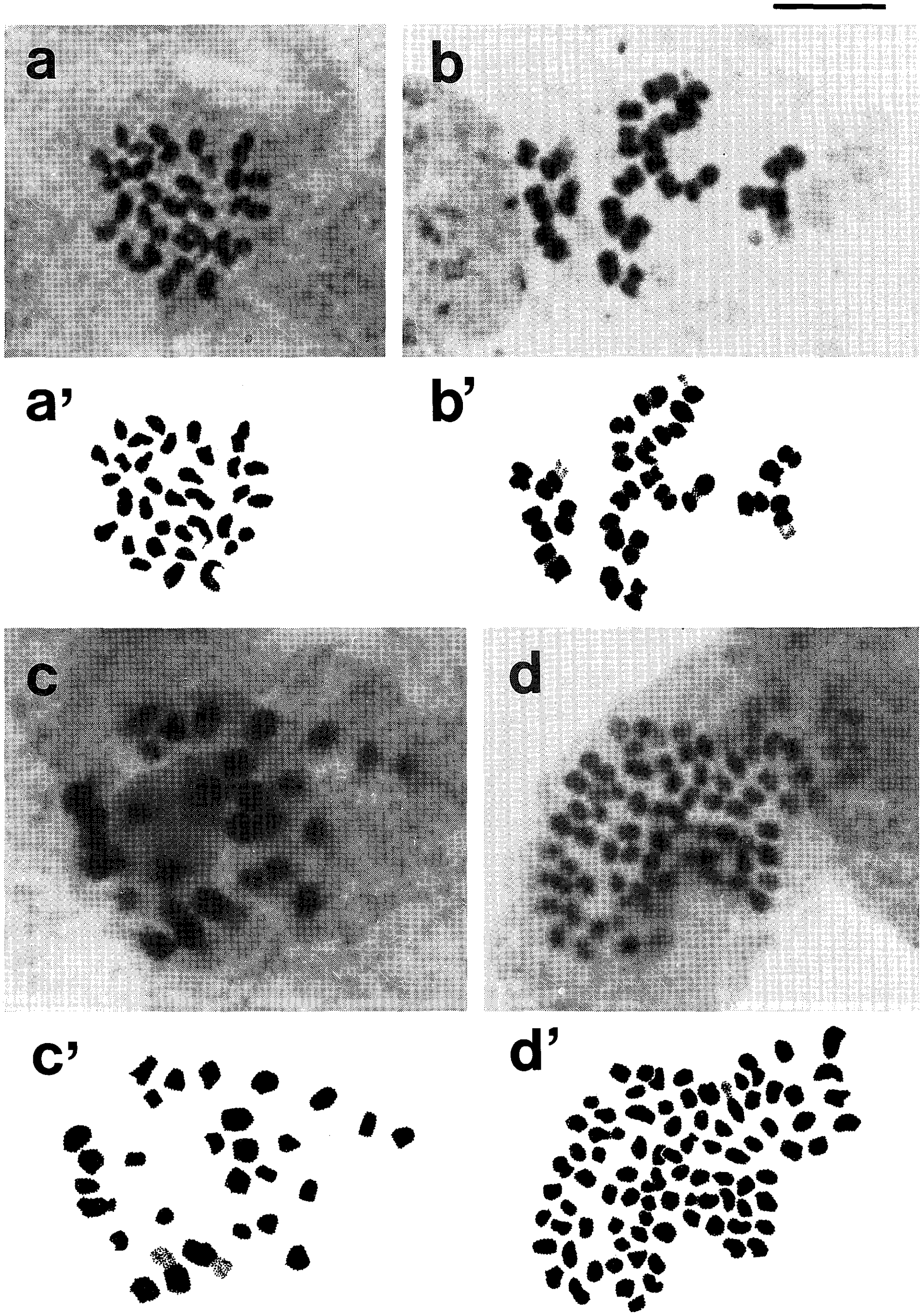

Fig. 1. Chromosomes of Himalayan *Sedum*. a, a′: *S. filipes*, 2n = 34 (voucher specimen no. 8320517). b, b′: *S. triactina*, 2n = 24 (Bhutan, no. 9361543). c, c′: *S. multicaule*, 2n = 28 (no. 8320511). d, d′: *S. multicaule*, 2n = 84 (no. 8520393). a′–d′: drawings based on the photographs. Sacle bar = 5 μm.

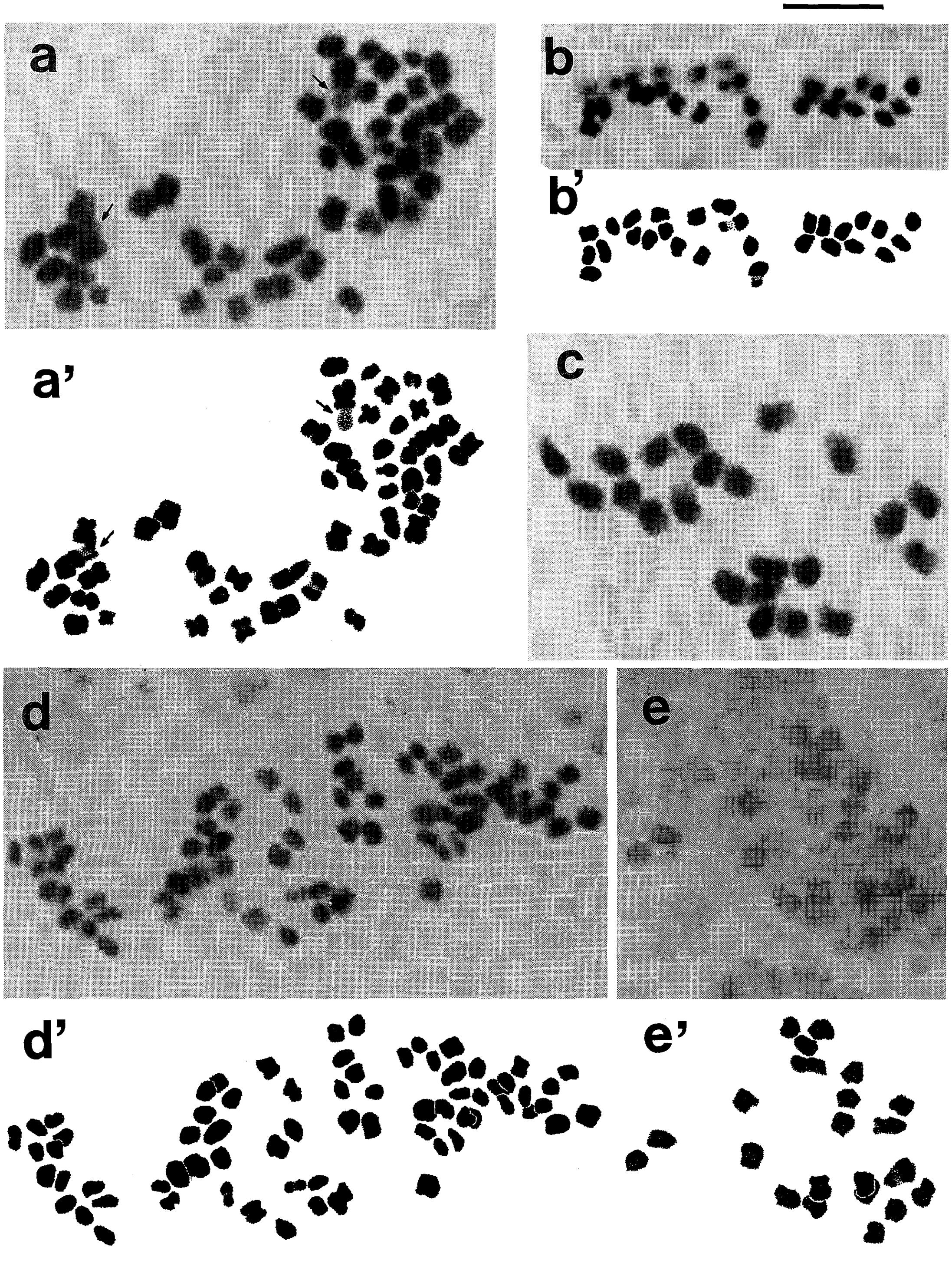

Fig. 2. Chromosomes of Himalayan *Sedum* and *Sinocrassula*. a, a′: *Sedum henrici-roberti*, 2n = 48 (voucher specimen no. 8520331); arrows show one pair of large submetacentric chromosomes with satellites. b, b′: *S. magae*, 2n = 28 (no. 9151241). c: *S. oreades*, 2n = 22 (no. 9151153). d, d′: *S. trullipetalum*, 2n = 70 (no. 9710288). e, e′: *Sinocrassula indica*, 2n = 22 (no. 8330343). a′, b′, d′, e′: drawings based on the photographs. Scale bar = 5 μm.

Sinocrassula. This is the first report of the chromosomes of Himalayan *Sedum* and the genus *Sinocrassula*.

In *Sedum* the seven species are classified into four different sections in subgenus Sedum. *Sedum filipes* Hemsl., representative species of section Filipes, has white flowers and a few pairs of large round leaves, and ranges from Himalaya to East Asia. The chromosome number 2n = 34 of *S. filipes* is regarded as diploid with the basic chromosome number x = 17.

Sedum triactina A.Berger is classified in section Bracteata which is limited to Himalaya and southwestern China. The species always grows on rock surface in the summer moist forests. The chromosome number 2n = 24 of *S. triactina* is considered to be diploid with x = 12.

Sedum multicalule Wall. ex Lindl. is a representative species of section Japonica, and has a wide range of distribution from Kashmir to central China through Himalaya, southern Tibet, Burma, and southwestern China. The species is rather variable, often vigorous, abundant in exposed situations like rock crevices, stone walls, and stone roofs along trails or roads. The plants with 2n = 28 are thought to be the diploid race while those with 2n = 84 are hexaploid. The basic chromosome number is estimated as x = 14 and is rare among the species in section Japonica. The hexaploid plants are vigorous and occur in exposed and disturbed places. The diploid plants have slender flowering stems and leaves, and occur in shaded mossy rocks in forests. These two cytoraces can be recognizable as varieties within a single species.

The species of section Oreades are characterized by the yellow petals and orthocarpic ovaries (that is, without the ventral swelling), and are diversified in higher elevations in the region from Himalaya to southwestern China, particularly in Yunnan and Sichuan. *Sedum oreades* (Descne.) R.-Hamet is the most common species having the widest range from Kashmir to Sichuan through Himalaya, Tibet, and Burma. The chromosome number 2n = 22 found in *S. oreades* is regarded as diploid with x = 11 and probably the lowest somatic chromosome number in sect. Oreades. In spite of having the widest range of distribution in sect. Oreades, the cytological and morphological variations of *S. oreades* are extremely narrow and has no geographical tendency nor cline. In Nepal *S. oreades* grows commonly in waste unstable exposed banks of stream sides in the alpine zone.

Sedum trullipetalum Hook.f. & Thoms. of sect. Oreades is well characterized by the broadly trullate petals with narrow claw and the moss like habit, and widely distributed in Sino-Himalayan region from Kumaun to southwestern China through Nepal, Bhutan, sohthern Tibet, Assam, and Burma. The chromosome number, 2n = 70, counted in this study, is considered as that showing high polyploidy. We examined the pollen fertility from the flowers of all the six voucher specimens of *S. trullipetalum* listed in Table 1. In all the voucher specimens the stamens were normal in shape, but the pollen grains in the anthers were abortive and not well-stainable by lactophenol-cotton blue, and the pollen fertility was estimated as 0 %. This suggests that the chromosome number, 2n = 70, shows odd polyploidy, and the plants having this chromosome number are considered to be either heptaploid (7x) with x = 10 or pentaploid (5x) with x = 14. Besides the voucher specimens, we also examined the pollen fertility from the herbarium specimens collected from the various places of Nepal and Bhutan (Table 2). As well as the voucher specimens, the pollen fertility, judging from the stainability by lactophenol-cotton blue, was also extremely low in all the specimens: 0 % to 5 % in 19 specimens, 6 % to 9 % in 5 specimens. It is likely from these results that the plants oc-

Table 2. Specimens examined for estimating pollen fertility of *Sedum trullipetalum* in Nepal and Bhutan

Locality	Collector and specimen no.	Pollen fertility**
N*: Janakpur zone; Rolwaling Khola, Beding-Na (4050 m)	Ohba et al. 8320632 (TI)***	0 %
N: Janakpur zone; Rolwaling Khola, Beding-Na (4050 m)	Ohba et al. 8331798 (TI)	8 %
N: Janakpur zone; Rolwaling Khola, Beding (3600 m)-Na (4050 m)	Ohba et al. 8320630 (TI)	7 %
N: Janakpur zone; Rolwaling Khola, Beding (3600 m)g-Na (4050 m)	Ohba et al. 8351289 (TI)	1 %
N: Janakpur zone; Rolwaling Khola, Na (4050 m)-Yalung Kharka (4750 m)	Ohba et al. 8331911 (TI)	2 %
N: Janakpur zone; Rolwaling Khola, Na-Rinaldung (4040 m)	Ohba et al. 8320633 (TI)	1 %
N: Janakpur zone; Rolwaling Khola, Na (4050 m)-Rinaldung (4300 m)	Ohba et al. 8331839b (TI)	3 %
N: Janakpur zone; Rolwaling Khola, Na (4050 m)-Rinaldung (4300 m)	Ohba et al. 8351318 (TI)	1 %
N: Janakpur zone; Khare Khola, Pam Lhang (4300 m)-Bitta Kharka (4100 m)	Ohba et al. 8351402 (TI)	3 %
N: Janakpur zone; Khare Khola, Pam Lhang (4300 m)-Bitta Kharka (4100 m)	Ohba et al. 8331951 (TI)	1.5 %
N: Sagarmatha zone; Shorong Himal, Thosa Kharka-Beni Kharka (4000 m)	Ohba et al. 8520291 (TI)***	0 %
N: Sagarmatha zone; Shorong Himal, Beni Kharka (3950 m)	Ohba et al. 8520300 (TI)***	0 %
N: Sagarmatha zone; Hinku Valley, Rangdu Kharka-Horsola Kharka (3820 m)	Wakabayashi et al. 9710192 (TI, MAK)***	0 %
N: Sagarmatha zone; Hinku Valley, Khare-Tangnag (4400 m)	Wakabayashi et al. 9710288 (TI, MAK)***	0 %
N: Sagarmatha zone; Hinku Valley, Khare-Tangnag (4300 m)	Wakabayashi et al. 9710287 (TI, MAK)***	0 %
N: Sagarmatha zone; Hinku Valley, Tangnag-Dig Kharka (4400 m)	Wakabayashi et al. 9710234 (TI, MAK)	0 %
N: Sagarmatha zone; Hinku Valley, Tangnag-Mosom Kharka (4070 m)	Wakabayashi et al. 9710305 (TI, MAK)	6 %
N: Sagarmatha zone; Hinku Valley, around Tangna	Miyamoto et al. 9584224 (TI)	5 %
N: Sagarmatha zone; Hinku Valley, Tangna-Samakang Kharka (3920 m)	Miyamoto et al. 9584237 (TI)	9 %
N: Bagmati zone; Singum Gompa-Gosain Kund (3600 m)	Kanai, Hara & Ohba 727279 (TI)	3 %
N: Bagmati zone; Gosain Kund (3920 m-4450 m)	Malla & Kanai 15096 (TI)	3 %
N: Dhaulagiri zone; Dolpo Dist., Tango Pass (3696 m)	Tabata et al. 3568 (TI)	1.5 %
N: Kosi zone; Arn Valley, Barun Khola, N.W. of Num (12000 ft)	Stainton 1658 (TI)	8 %
N: Mechi zone; Topke Gola (3600 m)-Jalang Chhyongo (4300 m) (3800 m)	Ohba 772621 (TI)	0 %
N: Around Lamni Nama (4500 m)	Ohba 772614 (TI)	0 %
N: East of Chalike Pahar (14000 ft.)	Stainton, Sykes & Williams 4524 (TI)	0 %
B: Chomolhari, Chakan	Nishioka s. n. (6 Sept. 1966) (TI)	0 %
B: Wangdi Phodrang Dist.; Tsonsothang (4200 m)	Miyamoto 9361638 (TI)	3 %
B: Wangdi Phodrang Dist.; Chukarpo (4300 m)-Chebisa (3690 m)	Miyamoto 9361653 (TI)	4 %
B: Paro Dist., Gunitshawa (2700 m)-Mem La (4140 m)-Sye Tombu (3980 m)	Suzuki et al. 9121018 (TI)	0 %

* N: Nepal, B: Bhutan. **pollen fertility estimated by stainability of cotton blue. *** voucher specimens for chromosome number counted as 2n = 70.

curring in Nepal and Bhutan are mostly odd polyploids with 2n = 70 and reproduced by apomixis. For tracing the origin of the polyploidy giving rise to such reproductive mode in the Himalayas, it may need to cytologically analyze many populations of *S. trullipetalum* occurring also in the alpine regions of Tibet and southwestern China.

Sedum henrici-roberti R.-Hamet of sect. Oreades is distributed in Nepal Himalaya and Tibet, and one of the representatives occurring in the subnival zone in elevations beyond 4500 m, and grows on unstable exposed gravely slopes. The chromosome number 2n = 48 is considered to be tetraploid with x = 12, but may be diplodized because of the presence of only one pair of large submetacentric chromosomes with satellites. *Sedum magae* R.-Hamet distributed in Kumaun to Nepal Himalaya is also representative in the subnival zone, and may be allied to *S. henrici-roberti*, but differs mainly in having narrowly obtrullate petals from the latter species having subulate to lanceolate petals. The chromosome number 2n = 28 of *S. magae* is considered to be diploid with x = 14, and this basic number is different from that of *S. henrici-roberti*.

In sect. Oreades, at least, three or four different basic chromosome number, x = 11, 12, 14, and probably 10 are found, suggesting that sect. Oreades is cytologically diverse.

Sinocrassula is the mono- or oligotypic genus distributed in the Sino-Himalayan region, and characterized by the succulent rosulate monocarpic features with a terminal inflorescence. The genus is considered to have, at least, the basic chromosome number $x = 11$, although it needs to examine more populations, especially those in south-western China where the plants are diversified and several species and varieties have been known (Fu and Fu 1984).

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若林三千男[a], 大場秀章[b]: **東部ヒマラヤ産マンネングサ属7種および *Sinocrassula indica*（ベンケイソウ科）の染色体数**

東部ヒマラヤに産するマンネングサ属の *Sedum multicaule* (Japonica節), *S. triactina* (Bracteata節), *S. filipes* (Filipes節) と Oreades節の4種, *S. trullipetalum*, *S. oreades*, *S. magae*, *S. henrici-roberti*, および *Sinocrassula* 属の1種 *S. indica* の染色体の観察を初めて行った. 中期染色体の大きさはどの種においても小さく, $0.6 - 2\ \mu\mathrm{m}$ であった. 染色体数の結果は Table1 に示されているが, マンネングサ属では $2n = 22$ (*S. oreades*), 24 (*S. triactina*), 28 (*S. multicaule, S. magae*), 34 (*S. filipes*), 48 (*S. henrici-roberti*), 70 (*S. trullipetalum*), 84 (*S. multicaule*) が, *Sinocrassula indica* では $2n = 22$ が見出された. $2n = 22$, 24, 28, 34 はそれぞれ基本数を異にする2倍体と考えられる. *S. henrici-roberti* の $2n = 48$ は $x = 12$ の4倍体とも考えられるが, 核形態からみると2倍体化していると推定される. *S. multicaule* では種内倍数性が認められ, $2n = 28$ は2倍体, $2n = 84$ は6倍体であろう. この両者は生育環境も異なり, 形態的差異も明瞭である. $2n = 70$ と確認された *S. trullipetalum* の証拠標本の花粉稔性はどれも0%であり, また東京大学標本室に所蔵されている採集標本（ネパール, ブータン産）のすべてについても花粉稔性は極端に低いものであった (Table2). このことから, 東部ヒマラヤに生じている *S. trullipetalum* 植物のほとんどは $2n = 70$ の奇数倍数体と推定され, 繁殖手段はアポミクシスによるものと考えられる. このような倍数性の起源を追跡するためには中国西南部やチベットなどの高山帯に分布する集団についてもさらに細胞学的な解析を進めていく必要があろう.

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